MEASUREMENT OF ANTIBACTERIAL ACTIVITY on DUCOTONE SILVER -044251

Quotation: PO9YPH200279-01

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Test Item: DUCOTONE SILVER -044251

Study Director: ..... Released on: November 4th, 2020
(F. Faccioli)

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the studies described in this report have been conducted under my supervision and in compliance with the following standards of Good Laboratory Practice:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organisation for Economic Co- Operation and Development, Paris 1998.
- Legislative decree n. 50 of March the 2nd, 2007. Enforcement of Community Directives 2004/9/CE and 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86 of April the 13th, 2007).
- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments.
- Certification N. 2019/25 released by the Italian Ministry of Health on September 12th 2019 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

There were no circumstances that may affect the quality or integrity of the study.

Study Director
(F. Faccioli)

November 9th, 2020
Date

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved Study Plan and the Standard Operating Procedures of Eurofins Biolab S.r.l.
The study and/or the test facility were periodically inspected by the Quality Assurance unit according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance unit, personnel independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections have been carried out and reported to the Director of the Study and to Eurofins Biolab's S.r.l. Management:

QAU INSPECTIONS	
PHASE	**DATE**
Experimentation: -Audit process-based -Audit study-based	 October 18th-20th, 2018 //
Documentation: - Study Plan - Amendment N.1 to Study Plan - Raw data - Final report	 June 09th, 2020 July 30th, 2020 November 05th, 2020; November 09th, 2020 November 05th, 2020; November 09th, 2020

This report accurately reflects the raw data.

Jessica Nava
QA GLP
(Jessica Nava)

November 09th, 2020
Date

SUMMARY

The aim of this study is to determine the Antibacterial Activity against *Staphylococcus aureus* ATCC 6538P and *Escherichia coli* ATCC 25922 .

To this purpose, the Sponsor has provide to Eurofins Biolab S.r.L. specimens in tartan coated "DUCOTONE SILVER -044251 " and "DUCOTONE SILVER - 044251 (NON TRATTATI)" as control.

Three specimens of 5.0 × 5.0 cm square samples of the treated and untreated control have been prepared for each strain and time point tested (t_0 and 24hours). Separately for each test strain, 0,4 ml of standardized culture at 2,5-10×10^5 cells/ml has been added to the specimen then the inoculum has been covered and gently pressed down with a 40x40 mm film so that the test inoculum spreads to, but does not leak beyond, the edges of the film.

The specimens inoculated have been incubated at 35±1°C, 90% RH.

At t_0 and after the specified contact time (24 hours), viable microorganisms have been enumerated by pour plate method on TSA at 35±1°C for 44±4 hours; then bacterial colonies from each dilution series have been counted and recorded and the Logarithmic reduction of bacteria from treated versus untreated samples at contact time has been calculated.

On the basis of obtained results, interpreted according to ISO 22196:2011, it can be stated that the Test Item "DUCOTONE SILVER - 044251, **has antibacterial activity,** in experimental condition adopted.

INTRODUCTION

This study has been carried out at the Test Facility Eurofins Biolab S.r.l. on behalf of the Sponsor.

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Measurement of antibacterial activity	July 30th, 2020	November 06th, 2020	V. Bellomo F. Faccioli

On July 30th 2020, an Amendment to Study Plan was issued in order to introduce different specimens requested to Sponsor due porous nature of specimens previously prepared that were not appropriate to standard recovery.

For this reason, in order to avoid absorption of inoculum, specimens on tartan as substrate for test item and untreated control have been provided by Sponsor.

Test has been performed on these last sent specimens.

On the basis of nature of specimens, additional verification of recovery at t_0 on treated specimens has been done.

REFERENCE

ISO 22196:2011 - Measurement of antibacterial activity on plastics and other non-porous surfaces

FILING

The Study Plan, the Final Report, Amendments (if present) and all raw data are filed in the archives of Eurofins Biolab S.r.l. for 10 years after the issuing of the Final Report.

At the end of the study the residual sample has not been kept because it was completely used for the test.

At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the documents/products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the GLP Test Facility Eurofins Biolab S.r.l.

TEST ITEM IDENTIFICATION

The test product consists of a Biocide and Antimicrobials.

Product	DUCOTONE SILVER -044251
Code	Not Provided
Composition	Not Provided
Stability	2 years
Storage	Room Temperature, protected from light
Hazard information	See SDS for details

ANALYZED SAMPLE

The analysed sample, representative of the test item, consists of squared specimen of tartan coated with test item.

Batch	4003001957ys
Manufacturing date	03/2020
Expiry date	03/2022
CoA	Not Provided
Parcel registration number	1. IP-LV-2020136-ACY 2. IP-LV-2020174-AGJ 3. IP-LV-2020196-ADL
Receiving Date	1. 15/05/2020 2. 22/06/2020 3. 14/07/2020
Material Aliquot Number	1. LV-MAT-FOV7-20-140-0983:a 2. LV-MAT-FOV7-20-174-0A94:a 3. LV-MAT-UN1P-20-202-0768:a*

***Analysed**

REFERENCE ITEM

The analysed Reference Item, consists of squared specimen of tartan used as substare for negative control (untreated).

Product	DUCOTONE SILVER -044251 (NON TRATTATI)
Parcel registration number	1. IP-LV-2020136-ACY 2. IP-LV-2020174-AGJ 3. IP-LV-2020196-ADL
Receiving Date	1. 15/05/2020 2. 22/06/2020 3. 14/07/2020
Material Aliquot Number	1. LV-MAT-FOV7-20-140-0984:a 2. LV-MAT-FOV7-20-174-0A94:a 3. LV-MAT-UN1P-20-202-0769:a*

*Analysed

The test item and the information concerning the test item were provided by the Sponsor.
All data related to the test item are under the responsibility of the Sponsor and have not been verified by the test facility.

Experimental Report - Determination of the antibacterial Activity

EXPERIMENTAL DESIGN

Test has been performed on three specimens treated with test item and on three control specimens for t_0 and at the contact time (24h).

EXPERIMENTAL CONDITIONS

ASSAY SYSTEM

Both of the following species of bacteria have been used:
- *Staphylococcus aureus* ATCC 6538P
- *Escherichia coli* ATCC 8739

Conservation

The bacterial strains were kept frozen; before use, they were transplanted on TSA slants and kept in a refrigerator at 5°C ± 3°C.

Preparation of the bacterial suspensions

The bacterial strains have been transplanted on Tryptone Soy Agar (TSA) slants twice consecutively and incubated at 35±1°C for 16-24 hours.

CULTURE MEDIA , REAGENTS

The validity of media and reagents has been verified before starting the analyses.
- Phosphate buffer solution (PBS)
- Suspension medium: 1/500 nutrient broth (1/500 NB):
  Nutrient Broth (NB)
  Water for injection (WFI)
- Neutralizer CEN
- Tryptone soy Agar (TSA)

EQUIPMENT

The validity of instruments and equipment has been assured following internal procedures before starting the analyses.
Standard microbiology laboratory equipment has been used and in particular:
- Laminar flow filtered work area
- Spectrophotometer
- Water bath
- Micropipettes
- Climate Chamber 35°±1°C, RH>90%

MATERIALS

Cover film that is 0,05-0,10 mm of thick, made of polyethylene has been used.

PROCEDURE

Preparation of test inoculum
Within two hours from the beginning of the test, one loop of the test bacteria has been transferred into 20 ml of 1/500 NB and evenly dispersed.
The suspension has been adjusted to a concentration of about 2.5 $x10^5$-$10x10^5$ cfu/ml, with a target concentration of $6x10^5$ cells/ml, concentration has been verified by pour plate method on TSA at 35±1°C for 40-48 hours.

Test Specimen
Flat of about 25 cm^2, not more than 10 mm in thickness specimens, treated with test item and untreated have been provided by Sponsor.

Inoculation of test specimens
Each test specimen has been placed into a separate sterile Petri dish.
0,4 ml of the test inoculum prepared have been inoculated onto the surface of specimens.
The test inoculum has been covered with a 40 x 40 mm film, that has been gently press down in order to the test inoculum spreads to, but does not leak beyond, the edges of the film.

Incubation of the inoculated test specimens
The Petri dishes with the inoculated test specimens have been incubated at 35±1°C, RH ≥90% for 24±1h.

Recovery of bacteria from test specimens
Test specimens immediately after inoculation
Immediately after inoculation, half of untreated and treated the specimens have been treated by adding 10 ml of Neutralizer and using an appropriate mechanical agitation (stomaching).

Test specimens after incubation
After contact time, all residual incubated specimens have been processed at the same of t_0.

Determining the viable bacteria count by the pour plate culture method
Viable bacteria has been enumerate by performing four 10-fold serial dilutions in PBS and 1 ml of each dilution (Full, -1,-2, -3, -4) has been plated in twice.
About 15 ml of TSA has been poured into each plate, swirled gently to disperse the bacteria, then the plates have been incubated inverted at 35±1°C for 40-48h.
After incubation, the number of colonies in plates has been recorded.

CALCULATION
For each test specimen, the number of viable bacteria recovered has been calculated according with following equation:

$$N = (100 \times C \times D \times V)/A$$

where
N is the number of viable bacteria recovered per cm^2 per test specimen;
C is the average plate count for the duplicate plates;
D is the dilution factor for the plates counted;
V is the volume, in ml, added to the specimen;
A is the surface area, in mm^2, of the cover film.

The geometric mean of the number of viable bacteria recovered for each set of test specimens has been calculated and this value expressed to two significant figures.

VALIDITY CONDITION

1) The logarithmic value of the number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall satisfy the following requirement:

$$(L\text{max} - L\text{min})/(L\text{mean}) \leq 0{,}2$$

where
- *L*max is the Log of the maximum number of viable bacteria found on a specimen;
- *L*min is the Log of the minimum number of viable bacteria found on a specimen;
- *L*mean is the Log of the mean number of viable bacteria found on the specimens.

2) The average number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall be within the range $6{,}2\times10^3$ cells/cm^2 to $2{,}5\times10^4$ cells/cm^2.

3) The number of viable bacteria recovered from each untreated test specimen at 24h shall not be less than $6{,}2\times10^1$ cells/cm^2.

When the three conditions are satisfied, the test is deemed valid. If any of these conditions are not met, the test is not considered valid and the specimens shall be retested.

CALCULATION OF THE ANTIBACTERIAL ACTIVITY

When the test is deemed valid, the antibacterial activity is calculated using following formula

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

where
- R is the antibacterial activity;
- U_0 is the average of Log of cells/cm^2, recovered from the untreated test specimens immediately after inoculation;
- U_t is the average of Log of in cells/cm^2, recovered from the untreated test specimens after 24 h;
- A_t is the average of Log of cells/cm^2, recovered from the treated test specimens after 24 h.

ANTIBACTERIAL EFFECTIVENESS CRITERIA

The value of the antibacterial activity can be used to characterize the effectiveness of an antibacterial agent. According to ISO 22196:2011, the antibacterial-activity values used to define the effectiveness shall be agreed upon by all interested parties.

RESULTS

All assay validity criteria have been satisfied.
In the following tables results obtained for each test strain.

Staphylococcus aureus ATCC 6538P

STRAIN		RESULT (cfu/ml)	Bacterial concentration $2,5 \times 10^5 \leq x \leq 10 \times 10^5$ cfu/ml	RESULT (cfu/inoculum)
A38	S. aureus ATCC6538P	8,8E+05	COMPLIES	3,5E+05

Contact time	Specimen	1 (cfu/ml)	2 (cfu/ml)	3 (cfu/ml)	Geometric mean (ufc/ml)
t0	Untreated	2,44E+04	2,46E+04	2,61E+04	2,50E+04
	Treated	2,59E+04	2,75E+04	2,87E+04	2,73E+04
t24	Untreated	8,45E+04	9,65E+04	9,30E+04	9,12E+04
	Treated	< 1,00E+00	< 1,00E+00	< 1,00E+00	< 1,00E+00

Contact time	Specimen	1 (ufc/cm^2)	2 (ufc/cm^2)	3 (ufc/cm^2)	Geometric mean (ufc/cm^2)
t0	Untreated	2,44E+04	2,46E+04	2,61E+04	2,50E+04
	Treated	2,59E+04	2,75E+04	2,87E+04	2,73E+04
t24	Untreated	8,45E+04	9,65E+04	9,30E+04	9,12E+04
	Treated	< 1,00E+00	< 1,00E+00	< 1,00E+00	< 1,00E+00

ASSAY VALIDITY CRITERIA	*1*	The Log value (L) of cfu/ml recovered immediately after inoculation from the untreated test specimens (NT t_0) shall satisfy the following requiremet: (Lmax – Lmin)/(Lmean) ≤ 0,2	COMPLIES
	2	The average number of viable microraganisms recovered immediately after inoculation from the untreated test specimens (NT t_0) shall be within the range $6,2 \times 10^3$ to $2,5 \times 10^4$ cells/cm^2.	COMPLIES
	3	The number of viable microrganisms recovered from each untreated test specimen after incubation for 24 h (NT t_{24h}) shall not be less than $6,2 \times 10^1$ cells/cm^2.	COMPLIES

Contact time	Specimen	1 (Log $_{ufc/cm2}$)	2 (Log $_{ufc/cm2}$)	3 (Log $_{ufc/cm2}$)	Log $_{ufc/cm2}$
t0	Untreated	4,39	4,39	4,42	4,40
	Treated	4,41	4,44	4,46	4,44
t24	Untreated	4,93	4,98	4,97	4,96
	Treated	< 0,00	< 0,00	< 0,00	< 0,00

Strain	Log reduction	% Reduction
S. aureus ATCC6538P	> 4,96	> 100,00

Escherichia coli ATCC 8739

STRAIN		RESULT (cfu/ml)	Bacterial concentration $2,5 \times 10^5 \leq x \leq 10 \times 10^5$ cfu/ml	RESULT (cfu/inoculum)
B5	E. coli ATCC8739	8,8E+05	COMPLIES	3,5E+05

Contact time	Specimen	1 (cfu/ml)	2 (cfu/ml)	3 (cfu/ml)	Geometric mean (ufc/ml)
t0	Untreated	2,03E+04	2,58E+04	2,80E+04	2,45E+04
	Treated	2,81E+04	3,45E+04	3,55E+04	3,25E+04
t24	Untreated	2,60E+06	2,28E+06	2,14E+06	2,33E+06
	Treated	< 1,00E+00	< 1,00E+00	< 1,00E+00	< 1,00E+00

Contact time	Specimen	1 (ufc/cm^2)	2 (ufc/cm^2)	3 (ufc/cm^2)	Geometric mean (ufc/cm^2)
t0	Untreated	2,03E+04	2,58E+04	2,80E+04	2,45E+04
	Treated	2,81E+04	3,45E+04	3,55E+04	3,25E+04
t24	Untreated	2,60E+06	2,28E+06	2,14E+06	2,33E+06
	Treated	< 1,00E+00	< 1,00E+00	< 1,00E+00	< 1,00E+00

ASSAY VALIDITY CRITERIA	*1*	The Log value (L) of cfu/ml recovered immediately after inoculation from the untreated test specimens (NT t_0) shall satisfy the following requiremet: (Lmax – Lmin)/(Lmean) ≤ 0,2	COMPLIES
	2	The average number of viable microraganisms recovered immediately after inoculation from the untreated test specimens (NT t_0) shall be within the range $6,2 \times 10^3$ to $2,5 \times 10^4$ cells/cm^2.	COMPLIES
	3	The number of viable microrganisms recovered from each untreated test specimen after incubation for 24 h (NT t_{24h}) shall not be less than $6,2 \times 10^1$ cells/cm^2.	COMPLIES

Contact time	Specimen	1 (Log $_{ufc/cm2}$)	2 (Log $_{ufc/cm2}$)	3 (Log $_{ufc/cm2}$)	Log $_{ufc/cm2}$
t0	Untreated	4,31	4,41	4,45	4,39
	Treated	4,45	4,54	4,55	4,51
t24	Untreated	6,41	6,36	6,33	6,37
	Treated	< 0,00	< 0,00	< 0,00	< 0,00

Strain	Log reduction	% Reduction
E. coli ATCC8739	> 6,37	> 100,00

DEVIATION

No deviation has been recorded from Study Plan.

CONCLUSIONS

On the basis of obtained results, interpreted according to ISO 22196:2011, it can be stated that the Test Item "DUCOTONE SILVER -044251, **has antibacterial activity,** in experimental condition adopted.